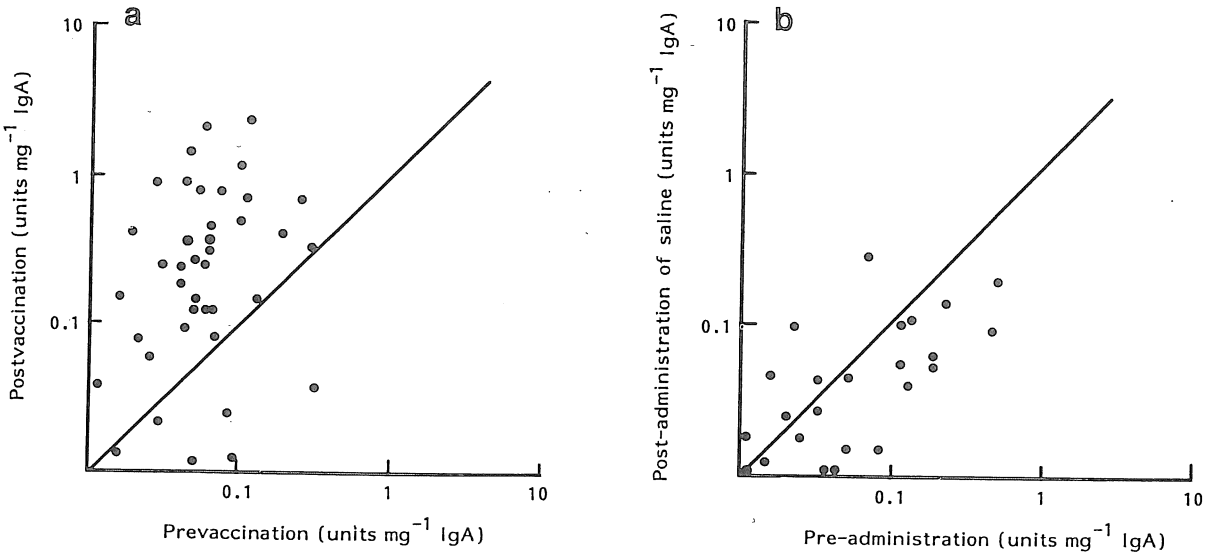

Figure 5 HA (A/Sichuan/2/87 (H3N2)) specific IgA antibody levels in nasal swab solutions before and after aerosol administration of the influenza vaccine (a) or of saline (b)

antigenic shift. When the base sequences of some specified regions of a RNA genome are changed to other base sequences, the mutation is called antigenic drift. Influenza virus vaccines used during the winter season of each year need to match with the subtypes of the influenza virus for that year. In the spring of every year WHO anticipates the subtypes of the influenza virus which will prevail during the following winter. Inactivated influenza vaccines are manufactured every summer using the subtypes of influenza virus recommended by WHO. In consequence, the combination of types and subtypes of the vaccine differs from year to year. Individuals need to receive influenza vaccine every year to avoid influenza virus infection. Therefore, any influenza vaccine to be used in humans in general needs to meet several conditions.

First, the vaccine has to be easy to administer and safe for human use, when given annually to the same individual. Some new vaccines have been tried in animals only and safety and efficacy in humans have not been tested at all. Inactivated split influenza vaccines given subcutaneously have been proven safe. Therefore, the same vaccine given in aerosol form without addition of any adjuvant may be one of the safest vaccines administered locally. We have administered approximately one thousand doses of aerosol inactived split influenza vaccine every year for the last 5 years. No adverse reactions to the vaccine were noted. In addition, local administration of a vaccine is easier than subcutaneous administration.

Secondly, a vaccine needs to be manufactured from the specified subtypes of influenza virus within a couple of months. With regards to split influenza vaccine in aerosol form, this condition is met fully because the aerosol form vaccine is exactly the same as the vaccine used subcutaneously.

Thirdly, it is preferable that the new vaccine induces local immunity. To meet this requirement, the vaccine, either live or inactivated, needs to be administered through the natural route of influenza infection. Along this line, a cold-adapted live attenuated reassortant vaccine was developed. A live influenza vaccine administered to the upper respiratory tract proliferates over the mucosal surface and induces local immune responses. Therefore, live influenza vaccine is effective only among people who do not have immunity to the subtype of the virus of the vaccine. Our study revealed that the inactivated influenza vaccine administered locally in the form of aerosol stimulated local antibody responses as well. In our study of 1988–89, most of the volunteers were already primed with A/Yamagata (H1N1), A/Fukuoka (H3N2), A/Sichuan (H3N2), and B/Nagasaki. Most of them already had detectable amounts of HA-specific local IgA antibodies against all four kinds of influenza viruses and serum HI levels $\geqslant$1:16. When the split influenza vaccines were administered intranasally in the form of aerosol, the vaccines had booster effects which were shown by rises in HA-specific local IgA antibodies, in serum HA-specific IgG, IgM and IgA antibodies, and in serum HI titres. Those results showed that the vaccines induced local immunoglobulin production and rises in serum-specific antibody levels even in the subjects who already had specific serum antibodies and/or local IgA antibodies to the subtypes of the virus of the vaccine.

Our results demonstrated that the route of administration of the inactivated split influenza vaccine influenced the type of immune response. Little change in serum HA-specific IgA antibody was noted after subcutaneous administration of inactivated influenza vaccine. On the other hand, rises in serum HA-specific IgA antibody could be observed after aerosol administration. Rises in local HA-specific IgA antibody were observed after aerosol administration of the inactivated influenza vaccine. The existence of a correlation between rises in local HA-specific IgA antibodies and rises in serum HA-specific IgA antibodies may suggest that local antigenic stimulation may result in production of specific IgA-producing cells.

Over the last decade, simultaneous epidemics of two or three different subtypes of influenza A virus and/or influenza B virus have been noted every year throughout the world. Therefore, most people have been primed with various subtypes of influenza virus. Aerosol administration of the inactivated split influenza vaccine can stimulate both local and systemic antibody responses even in those

who have been exposed to similar subtypes of the influenza virus before and have some degree of serum-specific antibody and/or local specific antibody to the subtype of the virus of the vaccine.

The first influenza virus was isolated from chickens with fowl plague in 1901. In 1933, Smith, Andrews and Francis succeeded in transmission of a human influenza virus to ferrets. Half a century has passed since the first influenza vaccine was administered to humans, yet no country in the world has ever succeeded in control of influenza epidemics by the use of vaccine given subcutaneously.

Epidemics of influenza are very hard to control. The only possible way to control epidemics is to obtain herd immunity. To achieve this, an increase in acceptance rate is needed and it may therefore be preferable to have a choice of several influenza vaccines. Because they can be easily administered to humans and because they induce production of local specific IgA antibodies which may be able to reduce chances of shedding of the specified influenza virus, intranasal administration of either live or inactivated influenza vaccines should have a place in the prevention of influenza epidemics in the very near future.

CONCLUSION

Systemic and local antibody responses to the topically administered and subcutaneously administered commercially available inactivated split influenza vaccines were investigated. Greater than fourfold rises in serum HI titres (A/Sichuan/2/87 H3N2) were observed in 87% (40/46) of the aerosol and in 93% (43/46) of the subcutaneous administration group. Greater than fourfold rises in serum HA (A/Sichuan/2/87 H3N2) specific IgG antibody were observed in 48% (22/46) and 37% (17/46), in specific IgM antibody were observed in 40% (17/43) and 20% (9/45) and in specific IgA antibody responses were observed in 58% (23/40) and 4% (2/46) of the aerosol and subcutaneous administration group, respectively. More than fourfold rises in local HA (A/Sichuan/2/87) specific IgA antibodies were observed in 28% (12/43) of the aerosol vaccine administration group and in 0% (0/24) of the control group who received aerosol saline in place of the vaccine.

Those observations suggest, first, that mucosal stimulation with inactivated influenza vaccine resulted in marked increase in local HA-specific IgA antibodies. Secondly, mucosal antigenic stimulation was necessary for serum HA-specific IgA responses. Thirdly, serum HA-specific IgA antibody levels can be used as indicators of the local antigenic stimulation. Finally, the inactivated influenza split vaccine, applied directly to the upper respiratory tract, is one of the most promising influenza vaccines for the foreseeable future.

It was concluded that aerosol administration of inactivated split influenza vaccine stimulated both local and systemic IgA antibody responses.

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REFERENCES

1 Immunization Practices Advisory Committee (ACIP). Prevention and control of influenza. *Morb. Mort. Week. Rep.* 1991, **40 RR-6**, 1–15

2 Ghendon, Y. The immune response of humans to live and inactivated influenza vaccines. *Adv. Exp. Med. Biol.* 1989, **257**, 37–45

3 Alford, R.H. Antibody in nasal secretions after intramuscular injections of influenza virus vaccine in persons with chronic bronchopulmonary disease. *J. Immunol.* 1969, **103**, 20–24

4 Clements, M.L. and Murphy, B.R. Development and persistence of local and systemic antibody responses in adults given live attenuated or inactivated influenza A virus vaccine. *J. Clin. Microbiol.* 1986, **23**(1), 66–72

5 Clements, M.L., Betts, R.F., Tierney, E.L. and Murphy, B.R. Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. *J. Clin. Microbiol.* 1986, **24**(1), 157–160

6 Ohta, K., Kuno-Sakai, H., Kimura, M., Oh, Y., Kim, R., Kumihashi, H. *et al.* Enzyme-linked immunosorbent assay of influenza specific IgA antibody in nasal mucus. *Acta Paediatr. Jpn* 1991, **33**, 617–622